Wavelength resolved laser-induced fluorescence emission of C$_6$F$_3$H$_3^{+}$ trapped in an ion cyclotron resonance cell

Brant Cage $^{a,1}$, Jochen Friedrich $^{a}$, Reginald B. Little $^{b}$, Yi-Sheng Wang $^{a,2}$, Melinda A. McFarland $^{a}$, Christopher L. Hendrickson $^{a,c}$, Naresh Dalal $^{c}$, Alan G. Marshall $^{a,c,*}$

$^{a}$ Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Drive, Tallahassee, FL 32310-4005, USA
$^{b}$ Department of Chemistry, Florida A&M University, Tallahassee, FL, USA
$^{c}$ Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32306, USA

Received 20 January 2004; in final form 19 May 2004
Available online 21 July 2004

Abstract

We have measured the wavelength resolved fluorescence emission laser-induced fluorescence (LIF) spectrum of C$_6$F$_3$H$_3^{+}$ confined in an open cylindrical ion cyclotron resonance Penning trap. Ion cyclotron resonant azimuthal quadrupolar excitation successfully mass-selects and traps the ions for 10 s for repeated laser interrogation, and establishes that the observed fluorescence is from C$_6$F$_3$H$_3^{+}$ ions. Electron-induced fluorescence (EIF) emission spectra of the same species were acquired for comparison. Vibrational structure was resolved for both LIF and EIF, and our assignments are consistent with prior literature values in the absence of ion trapping and high magnetic field (3 T). This work represents the first determination of wavelength resolved LIF emission spectra of organic ions in a Penning trap.

© 2004 Elsevier B.V. All rights reserved.

1. Introduction

Fluorescence resonance energy transfer (FRET) between a donor (emitter) and acceptor (absorber) group separated by distance, $r$, depends in large part on a dipole–dipole interaction proportional to $(1/r^6)$ [1,2]. Relative FRET values for various donor:acceptor pairs in a rigid macromolecule thus constrain the possible three-dimensional structures in much the same way as NMR $T_1$ values for pairs of spins in a macromolecule. FRET measurements for a given donor:acceptor pair covalently bound to opposite ends of a rigid molecular chain serve to calibrate the scale of this molecular ‘ruler’ [3]. The FRET technique is well established for investigation of macromolecular structures in aqueous solution [4].

The motivation for performing FRET experiments on gas-phase ions is high. For example, gas-phase protein ions produced by matrix-assisted laser desorption/ionization (MALDI) [5] or electrospray ionization (ESI) [6] are typically unsolvated. It is thus important to establish any differences between solution-phase and gas-phase structure: (a) to understand the effect of water on protein structure, and (b) to establish the validity of experiments designed to characterize the binding of ligands or other macromolecules to them in the gas phase (e.g., drug screening). A particular advantage of the gas-phase experiment is that it is possible to detect (and eliminate) contaminants, unwanted adducts, and com-
plexes from a sample in the mass spectrometer prior to measurement of laser-induced fluorescence (LIF). Finally, gas-phase FRET experiments are optimally performed in a trapped-ion mass analyzer (either Paul trap or Penning trap), to provide a large ion population and the capability to interrogate a given ion many times for more efficient LIF detection.

LIF of several atomic ions, including Mg−, Be+, Hg+, and Ba+ [7–12] has been measured in a Penning trap. Recently, fluorescence of molecular dye ions as a function of the number of ions in a Paul trap has been measured, as an important step toward gas-phase FRET of ions [13]. The first example of LIF of organic molecular ions in a Penning trap was the detection of the excitation spectrum of C6F6+ [14]. That result was followed by the first observation of fluorescence lifetimes (τ) of organic molecular ions stored in an ICR trap, demonstrating the feasibility of resolving wavelength overlapped fluorescence emission from chemically different species in a heterogeneous mixture (namely, an equimolar solution of C6F3H2 and C6F3H) by mass-selective ion cyclotron resonance [15].

To discriminate between donor and acceptor fluorescence in a FRET experiment, wavelength resolved emission spectra are desirable. Here, we demonstrate the first wavelength resolved LIF emission spectrum of organic ions mass-selected and stored in a Penning trap, thereby taking another step toward the synthesis of optical spectroscopy and mass spectrometry. We again chose fluorobenzenes as model compounds, because their radical cations fluoresce at much longer wavelength than do the parent neutrals. Allan and Maier [16] pioneered wavelength resolved LIF emission of fluorobenzene ions (C6F3H+) by electron ionization. As reviewed by Miller [17], the fluorobenzene cation ground state is formed by removal of an electron from the highest occupied π-orbital of the neutral, resulting in three electronic bound states denoted (from lowest to highest energy) as X, A, and B. Emission mainly corresponds to a π-π transition from the ground vibrational second excited electronic state to the quantum ladders of the various vibrational modes of the electronic ground state, generally denoted B → X. Later experiments [18–25] reported fluorescence excitation and emission spectra, revealing substantial vibrational detail, including an interesting Jahn–Teller distortion of the symmetric species, 1,3,5-trifluorobenzene. Our wavelength dispersed LIF emission spectra (see below) exhibit vibrational structure consistent with prior observations [16,18,22,24–26].

2. Experimental methods

1,3,5-trifluorobenzene (Aldrich Chemical Co., St. Louis, MO) is introduced into a homebuilt 3 T FT-ICR MS instrument [12,14] at a pressure of ~4×10−8 Torr (base pressure: 7×10−9 Torr) via a variable leak valve. Ions are produced by electron ionization (~45 V potential, several μA electron current) from an off-axis electron gun located in the fringefield of the magnet, and focused by the magnetic field to the central axis of a Penning trap. An open cylindrical trap consists of two end-cap cylinders at the front and back for confining ion axial motion (i.e., parallel to the magnetic field) and a sectioned middle cylinder for dipolar ICR excitation and detection and azimuthal quadrupolar excitation to axialize the ions during LIF measurements (see below), as described in previous publications [12,14].

For laser induced fluorescence (LIF) emission measurements, ions are trapped by applying a potential of 60 V to the front and back end caps of the Penning trap. The filament current is switched off after 1 s of ion accumulation (Fig. 1, top). To localize ions of a given mass-to-charge ratio, m/q, application of a three-dimensional axial quadrupolar electrostatic potential prevents ions from escaping axially parallel to the applied magnetic field. B. However, that potential also produces a radially outward-directed force that results in ion radial diffusion (and ultimate loss). It is thus necessary to introduce an additional azimuthal two-dimensional quadrupolar electric excitation (QE) at the ion cyclotron resonance frequency, ωc = qB/lm (Fig. 1, middle). In conjunction with a collision ‘buffer’ gas, the QE interconverts ion cyclotron and magnetron motion, and ion-neutral collisions rapidly damp the cyclotron radius (and thus the magnetron radius) to zero. The net effect is to squeeze the ion packet, leaving the ions centered (‘axialized’) along the magnetic field direction in a packet ~1 mm in diameter [27–31] of convenient size for repetitive laser interrogation. Specifically, helium buffer gas is pulsed into the vacuum chamber at ~1×10−5 Torr, and 1,3,5-trifluorobenzene radical cations are axialized by single-frequency QE at 368 kHz, 18.2 Vp-p. Slightly off-resonance axialization at 368 kHz (the C6H3F3+ ICR frequency was 354.9 kHz) proved to be more efficient than resonant axialization. All aspects of the ICR experiment are controlled by a MIDAS [32,33] data station.

A dye laser (Lambda-Physik Scanmate 2; Coumarin 120 dye, Ft. Lauderdale, FL) pumped by the third harmonic of a Nd:YAG laser (Continuum Surelite I, Santa Clara, CA) excited 1,3,5-trifluorobenzene ions at λexc = 448 nm (3 mJ/pulse) at a repetition rate of 10 Hz.
The LIF emission spectrum of electron-ionized 1,3,5-trifluorobenzene is shown in Fig. 4 and the EIF emission spectrum is shown for comparison in Fig. 5. Intensities are normalized to the highest-magnitude peak at 458.6 nm (LIF) and 457.4 nm (EIF). Despite the high counting noise (S/N) ratio [35], the minimum number of trapped C_{6}F_{3}H_{3}^{+} ions may be estimated as \(10^6\). The single peak at the ICR frequency of C_{6}F_{3}H_{3}^{+} (354.0 kHz) attests to the absence of contaminant or fragment ions. (The small feature at 708.1 kHz is a harmonic (i.e., twice the ICR frequency of C_{6}F_{3}H_{3}^{+}).)

For electron-induced fluorescence (EIF) experiments, a higher C_{6}F_{3}H_{3} pressure of \(10^{-4}\) Torr is maintained. The ions are not trapped, but rather produced and excited by impact of a continuous electron beam from the electron gun. The CCD is operated at a temperature of \(-20\) °C. It is internally gated at 10 Hz for 50 ms accumulation periods; i.e., the ion counting mode is disabled. The EIF spectrum (see below) comprises 1000 accumulations. It is corrected for the background fluorescence (1000 accumulations with the filament of the e-gun switched off), for a total accumulation period of 200 s.

3. Results and discussion

The LIF emission spectrum of electron-ionized 1,3,5-trifluorobenzene is shown in Fig. 4 and the EIF emission spectrum is shown for comparison in Fig. 5. Intensities are normalized to the highest-magnitude peak at 458.6 nm (LIF) and 457.4 nm (EIF). Despite the high counting threshold, the LIF spectrum shows several spikes attributed to single pixel artifacts due to thermal agitation. They were not removed from the spectrum except for two single points at 448.8 and 485.0 nm with a peak-to-noise ratio greater than 5. The smooth line in Fig. 4 represents a running 10 point average to guide the eye.
The fluorescence in Fig. 4 was pumped at 448 nm, corresponding to the transition from the ground vibrational ground electronic state to the ground $v_{13}$ vibrational mode (we use the mode numbering scheme adopted by Miller et al.) [19,36] of the second electronic excited state, determined from its laser-induced fluorescence excitation spectrum [23]. That transition was chosen for excitation because of its strong relative fluorescence intensity and spectral displacement from the wavelength region of interest ($\lambda > 457$ nm). Despite the background subtraction, a peak at the laser position (~448 nm) still appears in the spectrum, and may be attributed to imperfect subtraction due to scattered light intensity exceeding the fluorescence by a factor of > 10, as is the dip at ~478 nm attributed to an unidentified background peak that exceeds the fluorescence by a factor of > 5.

The spectrum in Fig. 4 shows three distinct features ascribed to fluorescence of C$_6$F$_3$H$_3^+$: a small peak at 452.9 nm, the main peak at 458.6 nm (S/N 50:1) and a peak at 469.4 nm. The accuracy of these peak positions is ±0.3 nm. Two further features are discernible at ~481 and ~496 nm. The small peak at 452.9 nm is assigned to the $\nu_{13} \rightarrow \nu''_{13}$ (j = 3/2) hot band transition from the singly excited vibrational mode 13 of the second electronic excited state, $v_{13}$, to the singly excited vibrational mode 13 of the ground electronic state, $v''_{13}$ (in agreement with $\lambda = 452.6$ from Sears et al. [26] (see [20] for discussion of the quantum number, $j$)). That vibrational mode corresponds to an asymmetric –C–C–C– bend that likely reduces the molecular symmetry from D$_{3h}$ to C$_2$ [20].

Previous measurements [21,22,24,26] (and EIF, see Fig. 5) report the origin transition, $\nu_0 \rightarrow \nu''_0$, from the ground vibrational second electronic excited state, $\nu'_0$, to the ground vibrational ground electronic state, $\nu''_0$, at ~457.5 nm. The main transition in Fig. 4 is significantly redshifted relative to this value by more than 1 nm, suggesting an assignment $\nu_{13} \rightarrow \nu''_{13}$ (j = 1/2) rather than $\nu_0 \rightarrow \nu''_0$ [26]. Because the most intense peak is at 458.6 nm (not the j = 3/2 peak observed by Sears), we assign the peak at 458.6 nm to an unresolved superposition of $\nu_0 \rightarrow \nu''_0$ (reported at 457.5 nm [26]), and $\nu_{13} \rightarrow \nu''_{13}$ (j = 1/2) (reported at 458.6 nm [26]) and possibly further weak transitions observed in the 457–462 nm region (see Fig. 5 and [18]). Moreover, we used a counting device for signal processing, leading to a distortion of relative intensities for signal intensities above one photon per acquisition and bin, thereby reducing the apparent intensities of large-magnitude peaks. Consequently, coalescence with less intense peaks is more pronounced. The peak at 469.4 nm is assigned to the $\nu_0 \rightarrow \nu''_0$ transition in agreement with literature values [22,25]. The observed peak positions, previous literature values, and assignments for the EIF spectrum in Fig. 4 are listed in Table 1.

The two remaining features cannot be assigned reliably due to low S/N ratio in that part of the spectrum as well as the artifact at ~478 nm. They do, however, qualitatively agree with the EIF spectrum.

The EIF spectrum (Fig. 5) shows also two distinct peaks: the origin band, located at 457.4 nm ($\pm 0.2$ nm; $\nu_0 \rightarrow \nu''_0$; S/N > 1 : 100) and a transition at 469.3 nm ($\pm 0.2$ nm; $\nu''_0 \rightarrow \nu''_{13}$). Furthermore, a shoulder at ~448 nm ($\nu_{13} \rightarrow \nu''_0$), a small peak at 452.7 nm ($\pm 0.5$ nm; $\nu_{13} \rightarrow \nu''_3$ (j = 3/2)), and two weak features at 478.9 ($\pm 0.5$ nm; $\nu''_0 \rightarrow 2\nu''_0$) and 494.8 ($\pm 0.5$ nm; $\nu_0 \rightarrow \nu''_{10}$) are discernible (see Table 2). The assignments are consistent with previously published spectra [16,18,22] to within experimental error. The small peak at 486.1 nm is attributed to a hydrogen Balmer line [16].

---

Fig. 4. Laser-induced wavelength resolved emission spectrum of C$_6$F$_3$H$_3^+$. Open circles: raw data; Solid line: 10-point moving average to guide the eye. The fluorescence was pumped at 448 nm.

Fig. 5. Electron-induced wavelength resolved fluorescence emission spectrum of C$_6$F$_3$H$_3^+$.
Table 1
LIF peak positions (nm) and assignments

<table>
<thead>
<tr>
<th>LIF</th>
<th>Assignment</th>
<th>Literature [26]</th>
</tr>
</thead>
<tbody>
<tr>
<td>448 nm</td>
<td>Laser</td>
<td></td>
</tr>
<tr>
<td>452.9</td>
<td>$v_{13}^{13} \rightarrow v_{13}^{13} (j = 3/2)$</td>
<td>452.6</td>
</tr>
<tr>
<td>458.6</td>
<td>$v_{13}^{13} \rightarrow v_{13}^{13} (j = 1/2)$</td>
<td>457.5, 458.6</td>
</tr>
<tr>
<td>469.4</td>
<td>$v_{0}^{13} \rightarrow v_{11}^{13}$</td>
<td>469.5</td>
</tr>
<tr>
<td>481</td>
<td>Not assigned</td>
<td></td>
</tr>
<tr>
<td>496</td>
<td>Not assigned</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
EIF peak positions and assignments

<table>
<thead>
<tr>
<th>EIF</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>~448</td>
<td>$v_{13}^{13} \rightarrow v_{0}^{13}$</td>
</tr>
<tr>
<td>452.7</td>
<td>$v_{13}^{13} \rightarrow v_{13}^{13} (j = 3/2)$</td>
</tr>
<tr>
<td>457.4</td>
<td>$v_{0}^{13} \rightarrow v_{10}^{13}$</td>
</tr>
<tr>
<td>469.3</td>
<td>$v_{0}^{13} \rightarrow v_{13}^{13}$</td>
</tr>
<tr>
<td>478.9</td>
<td>$v_{0}^{13} \rightarrow 2v_{13}^{13}$</td>
</tr>
<tr>
<td>494.8</td>
<td>$v_{0}^{13} \rightarrow v_{10}^{13}$</td>
</tr>
</tbody>
</table>

The LIF and EIF spectra show roughly the same structure, but with some differences. (i) Most obvious, both the resolution and S/N ratio of the EIF spectrum are higher. (ii) The intensity ratios of the LIF peaks are distorted due to discriminator effects as discussed above. (iii) The main peak of the LIF spectrum is apparently red shifted, presumably due to peak coalescence. (iv) The $v_{0}^{13} \rightarrow 2v_{13}^{13}$ transition cannot be identified in the LIF spectrum because of an apparent shift due to a background artifact.

Unfortunately, the electron impact generation of ions for the EIF example here cannot easily be applied to large, fragile, nonvolatile molecules such as proteins. Moreover, we were not able to trap and mass-select ions for EIF, due to the need for a high-intensity electron beam for that experiment. In any case, the present results convincingly demonstrate the feasibility of wavelength resolved LIF emission spectroscopy for trapped organic ions. The stage is now set to apply optical fluorescence excitation and emission spectroscopy as well as fluorescence lifetime measurements to mass-selected trapped gas-phase organic ions.

4. Conclusion

We have shown for the first time that wavelength resolved laser-induced fluorescence emission spectra are readily obtainable from mass-selected organic ions in a Penning trap. Specifically, we observe vibrational structure and a Jahn–Teller active $v_{13}$ vibrational mode of the electronic ground state for $C_6F_3H_9^+$, consistent with previous literature observations [22,24–26]. Azimuthal quadrupolar rf excitation counteracts the tendency for ions to diffuse and escape radially from the Penning trap, and enables repetitive laser interrogation of the same ions; ICR dipolar excitation/detection affords direct identification of the fluorescing species.

Three major forms of optical spectroscopy of organic ions mass-selected and trapped by ICR have been demonstrated by our group in the last two years: (i) LIF excitation spectroscopy [14] as a measure of the optical absorption spectrum, (ii) fluorescence lifetime [15] measurements that reflect fluorophore environments and macromolecular conformations [2]; and (iii) wavelength resolved LIF emission spectroscopy to reveal the quantum parameters of the involved electronic states. The S/N ratio (50:1) of the LIF emission spectrum suggests that donor–acceptor fluorescent labeling of proteins in solution [2], and recently reported in a quadrupole ion trap for gas-phase oligonucleotides [37], should be feasible for gas-phase proteins. Although the acquisition period for the wavelength resolved LIF emission experiment is long (~80 h), it can be reduced by use of a continuous-wave rather than 10 Hz pulsed laser excitation source, and fluorescence lifetime measurements require much less time than it takes to wavelength resolve the LIF emission.

Acknowledgments

The authors thank John P. Quinn for helpful discussions and technical support and Greg T. Blakney for computer support and assistance with MIDAS. Additionally, we thank the FSU Department of Chemistry and Biochemistry for partial assistance with the purchase of the ICCD camera. This work was supported by the NSF National High Field FT-ICR Facility (CHE-99-09502), Florida State University, and the National High Magnetic Field Laboratory in Tallahassee, FL.

References